A Distinct Genotype of XP Complementation group A: Surprisingly Mild Phenotype Highly Prevalent in Northern India/ Pakistan/ Afghanistan

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Short title: XP complementation group A with a mild phenotype

Abbreviations:

XP xeroderma pigmentosum

UVR ultraviolet radiation

NER nucleotide excision repair

Letter to the editor

Xeroderma pigmentosum (XP) is a rare inherited disorder of DNA repair. Affected individuals cannot repair ultraviolet radiation (UVR)-induced DNA damage, resulting in an increased skin cancer risk (Bradford *et al*., 2011), severe sunburn in approximately 50% of patients, (Sethi *et al*., 2013) and progressive neurodegeneration in approximately 30% (Kraemer *et al*., 1987; Totonchy *et al*., 2013). XP can result from defects in any of eight genes (XP-A to XP-G and variant), *XPA*-*XPG* being involved in nucleotide excision repair (NER) of DNA damage (Cleaver *et al*., 2009).

XP-A patients, deficient in XPA protein, usually have a severe phenotype, with exaggerated sunburn and early onset of progressive neurodegeneration, which results in death, usually in the second or third decade (Anttinen *et al*., 2008). XPA protein is required for damage verification in the NER pathway. Over 20 different mutations have been identified in the *XPA* gene (States *et al*., 1998; Takahashi et al., 2010). Many of the reported cases come from Japan because of a founder mutation (c.390-1G>C) carried by 1% of the Japanese population (Hirai *et al*., 2006; Satokata *et al.*, 1990). This results in abnormal splicing of mRNA and subsequent production of truncated, non-functioning XPA protein and the typically severe clinical phenotype.

Although a diagnosis of XP-A has usually been associated with poor prognosis, a number of XP-A patients, under long-term follow-up at the UK National XP Clinic, have a surprisingly mild phenotype. To examine this finding further, a detailed genotype-phenotype study in this cohort was conducted. Specifically, patients were examined by specialists, and underwent audiometry, nerve conduction studies, brain MRI scans, and neuropsychometric evaluations. Informed consent was obtained from all patients and this study was performed in accordance with protocols approved by the Research Ethics Committee of Guy's and St Thomas' Hospitals NHS Foundation Trust, London (reference 12/LO/0325).

Nineteen out of 90 patients under study at the UK National XP clinic were assigned to complementation group A (Table 1). Twelve of these, from eight consanguineous families, displayed a mild XP-A phenotype with no ocular surface disease, delayed onset or lack of skin cancer and normal neurological and neuropsychometric evaluations (Figure 1A-H). Their mean age at assessment was 32 years (age range 6 to 79 years) and mean age at clinical diagnosis was 28 years (range 4 to 46 years), significantly higher than the more severely affected XP-A group with progressive neurogeneration presenting as developmental delay and cognitive impairment, sensorineural hearing loss, microcephaly, neuropathy and cerebellar signs (Table 1). Remarkably, one of the patients, XP1CB, is aged 79 years without any XP-related neurological problems. He spent the first thirty years of his life in India working largely outdoors and was only diagnosed clinically aged 46 years. These 12 patients were all homozygous for the mutation c.555+8A>G, which has been previously reported by Sidwell *et al.,* 2006 in a 61 year-old Punjabi woman with no neurological problems. All 12 patients included here, as well as the case described by Sidwell *et al.* originate from a 950km stretch of land around the Northern India/ Pakistan/ Afghanistan borders, suggesting a founder effect present in this population (Figure 1I).

The c.555+8A>G mutation at the eighth nucleotide of intron 4 generates a new splice donor site and results in aberrant splicing of intron 4 and non-functional truncated XPA protein. However, there is a small amount of normally spliced mRNA (Sidwell *et al.,* 2006), which results in production of detectable residual normal XPA protein in immunoblots (Figure 1J). Comparison of the upper XPA band in lanes 9-12 with the calibration in lanes 1-6 suggests that 50 μg extract from the mild XP-A cells has the same as or less XPA protein than 2.5 μg normal extract (lane 2), indicating the presence of less than 5% of XPA protein in the mild XP-A cells. In contrast no protein is detectable in XP-A null cell line XP15BR (lanes 1 and 13). This residual protein carries out NER, consistent with the 5-15% of normal UDS found in these patients (Figure 1K). It has been shown that low levels of XPA protein, transfected into XPA-deficient cells, are able to significantly protect against DNA damage (Muotri *et al*., 2002). This most likely explains their normal neurological phenotype.

Interestingly, the sunburn reactions in this group were variable, despite all patients being of similar ethnicity. This may be explained by the fact that the very small amount of functioning XPA protein may not be sufficient to repair the high level of photoproduct accumulation after sun exposure, resulting in moderate sunburn severity. However endogenous neurological damage is likely to be generated continually, at a low rate and therefore the low level of functioning XPA protein may be sufficient to repair the damage as it forms, resulting in a normal neurological phenotype.

There has been a handful of other mild XP-A patients reported in the literature, although none as mild as our cohort. Four middle-aged Japanese XP-A patients presented with late onset neurological impairment and moderate sunburn reactions, without development of skin cancer (Takahashi *et al*., 2010). Their milder phenotype was attributed to frameshift mutations in exon 6 (c.689dupT p.(Arg231fs) in one patient, c.779delCinsTTCTT p.(Thr260fs) in the other three) producing truncated XPA proteins.

This study highlights the importance of genotype-phenotype correlations in XP, not only for diagnosis but also for prognosis and genetic counselling. In this paper we present 12 XP-A patients with variable sunburn reactions and normal cognitive and neurological phenotype; the largest number of mild XP-A patients reported to date. Although the milder skin phenotype may be to some extent related to their more pigmented Fitzpatrick skin types, in our cohort of patients, homozygous for c.555+8A>G, we are now able to give cautiously optimistic prognostic information with regards to lack of neurodegeneration and later onset of skin cancer. A diagnosis of mild XP-A should be considered in individuals with facial lentigines, originating from the borders of Northern India, Pakistan and Afghanistan, as early photoprotection can reduce further development of lentigines and potential skin cancers. Our findings, from a relatively small immigrant population in the UK, imply that there may be many such individuals in the area of origin, who are likely to be undiagnosed because of the mildness of symptoms and may suffer from excessive skin damage later in life.

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Figure legend

Figure 1. Face views of mild xeroderma pigmentosum complementation group A (XP-A) patients and map of origins, immunoblots of protein extracts probed with monoclonal antibody to XPA protein, and measurement of UDS in mild XP-A and control cell lines. (a) XP103BR: Seven-year-old girl with a few facial lentigines (distantly related to patients shown in e-g). (b) XP53BR: Eighteen-year-old man who presented with sunburn lasting 1 week and increased lentigines at exposed sites. (c, d) XP2PR, XP1PR: Two siblings aged 32 and 35 years, respectively, who developed facial lentigines at age 2 years (distantly related to patients shown in e-g). (e-g) XP89BR-S, XP88BR, XP89BR: Three siblings, currently aged 34, 36, and 43 years, respectively, who presented with increased facial lentigines and easy sunburn. (h) XP1CB: Seventy-nine-year-old man who developed lentigines at exposed sites at age 6 years. Of the seven other siblings in his family, four have XP. Until 30 years of age he had worked outdoors as a veterinarian in India, with high cumulative ultraviolet radiation exposure. He then moved to the United Kingdom and worked indoors as a pathologist until his retirement. He developed melanoma in situ on his left cheek at age 46 years and since then has developed 8 melanomas and >20 nonmelanoma skin cancers. He underwent a left hemicolectomy for a sigmoid colon adenocarcinoma Dukes B at age 55 years, followed by further surgery for mucinous adenocarcinoma Dukes B2 the following year. He subsequently developed keratoacanthomas and a sebaceous adenoma, leading to a diagnosis of Muir-Torre syndrome [mutation c.306G>T in MLH-1 (Thompson et al., 2014)] unrelated to his mutation in the XPA gene. (i) Origins of the eight families with mild

XP-A are indicated on the map. (j) Immunoblots of protein extracts probed with monoclonal antibody to XPA protein (BD Bioscience, Oxford, UK, #556453). Left: Calibration: The 50-μg protein extract is made up of the indicated quantities from normal 48BR cells and XPA-null XP15BR cells. Right: The 50 μg of extract from the indicated cells. The positions of the 50-kDa and 37-kDa markers are indicated between the panels. XPA protein, indicated with arrows, runs as two bands on either side of the 37-kDa marker. Normal XPA protein level of ≤5% is detected in the mild XP-A cases but not in XP15BR. (k) UDS measured by incorporation of 3H-thymidine into nondividing cells after ultraviolet-C irradiation with the indicated doses. From 5% to 15% of normal UDS is detected in cells from the mild XP-A patients but is undetectable in XP15BR. UDS, unscheduled DNA synthesis. All patients pictured here have provided written and oral consent for publication of these photographs.

Table 1. Summary of clinical features in XP-A patient cohort

XP number	Age (Sex)	Country of Origin	[a]Age at XP diagnosis	SSS	Cutaneous features	Developmental/ neuropsychometric and neurological evaluation	Age 1st mucocutaneous cancer (type)	Mutation in *XPA* gene
XP9BI	6 (F)	Pakistan	6	0	Lentigines	Normal		c.555+8A>G
XP103BR	7(F)	Pakistan	4	0	Lentigines	Normal		c.555+8A>G
XP53BR	18(M)	Pakistan	7	1	Lentigines / Photosensitivity	Normal		c.555+8A>G
XP121BR	25 (F)	Pakistan	25	1	Lentigines	Normal		c.555+8A>G
XP116BR	31(M)	India	31	1	Lentigines	Normal	31 (BCC)	c.555+8A>G
XP2PR	32(F)	Pakistan	32	1	Lentigines	Normal		c.555+8A>G
XP89BR-S	34(M)	Pakistan	33	2	Lentigines	Normal		c.555+8A>G
XP1PR	35(M)	Pakistan	35	2	Lentigines	Normal		c.555+8A>G
XP88BR	36(M)	Pakistan	31	3	Lentigines	Normal		c.555+8A>G
XP49BR	38(M)	Afghanistan	24	1	Lentigines / Photosensitivity	Normal		c.555+8A>G
XP89BR	43(F)	Pakistan	39	3	Photosensitivity	Normal		c.555+8A>G
XP1CA	79(M)	India	67	0	Lentigines	Normal	46 (MM)	c.555+8A>G
XP111BR	7(F)	Bangladesh	5	3	Lentigines	Abnormal		c.253C>T p.(Gln85TER)
XP57BR	14(F)	Bangladesh	1	1	Photosensitivity	Abnormal		c.640dupA p.(Met214fs)
XP80BR	14(F)	Somalia	8	3	Photosensitivity	Abnormal		c.314G>A p.(Cys105Tyr)
XP81BR	18(M)	Somalia	12	1	Photosensitivity	Abnormal		c.314G>A p.(Cys105Tyr)
XP15BR	22(M)	UK	0.5	3	Photosensitivity	Abnormal		c.266_267dupAA p.(Val90fs)
XP114BR	24(M)	Pakistan	22	3	Photosensitivity	Abnormal	22 (SCC)	c.682C>T p.(Arg228TER)
XP20BR	32(M)	Pakistan	13	3	Photosensitivity	Abnormal	22 (ocular CIN3)	c.682C>T p.(Arg228TER)

SSS: Sunburn severity score (Sethi *et al.,* 2013), BCC: basal cell carcinoma, MM: malignant melanoma, SCC: squamous cell carcinoma, CIN: conjunctival intraepithelial neoplasia.

[a]Age of diagnosis by cellular measurement of defective NER or identification of pathogenic mutation(s). Clinical diagnosis may have been earlier.